QUICK GUIDE TO TECHNICAL INSTRUCTIONS FOR USE AND CLEANING OF MD STAINER FOR IMMUNOHISTOCHEMISTRY (IHC)

IMPORTANT

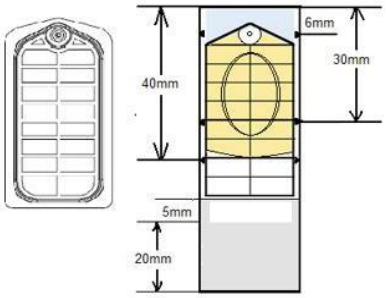
This is a quick guide for the use of MD Stainer, which include the most relevant information for the use, maintenance and cleaning of the equipment for Immunohistochemistry (IHC), as well as instructions for the preparation of tissue sections. For more detailed information, the user can consult the *User Manual*.

For the automated use of the equipment, the user must have been given a previous training by the equipment supplier.

STAINING AREA

In order to guarantee the quality and reliability of the staining, the sections must be prepared according to the following guidelines:

- In the picture below, **the position recommended for the tissue** is marked with a circle.
- The area highlighted in yellow shows the scope of the **staining area**. That is why the section position is important.



PREPARATION AND PRETREATMENT OF PARAFFIN-EMBEDDED SECTIONS

SECTION

Paraffin-embedded tissue sections must be **3 µm** thick and placed on the slide, respecting the **tissue placement area recommended** in the previous section.

Note: in order to obtain optimal results, it is recommended to prepare the sections a day in advance maximum. If they are made earlier, it is recommended to heat them for a maximum of 5 minutes so that they adhere to the slide correctly and left them at room temperature in a protected area until they are to be stained (maximum one week).

HEATING

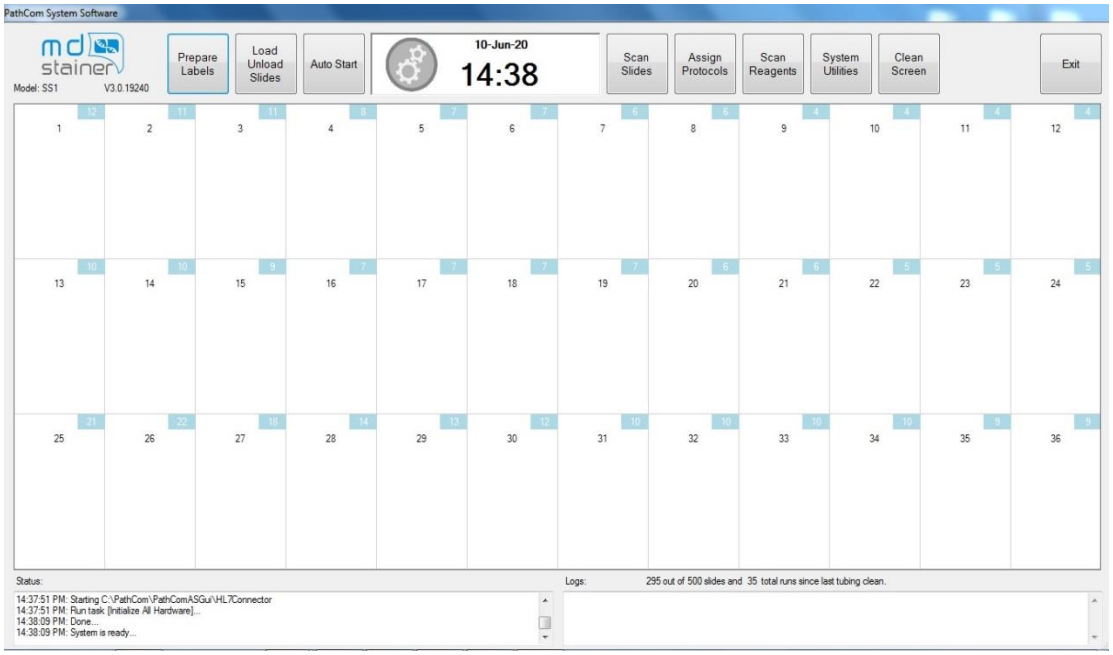
- The preparations must be heated right before introducing them into the MD-Stainer at **60ºC** for **at least 1 hour**. Following these guidelines, the sections will adhere correctly and most of the extra tissue paraffin will be eliminated.
- The preparations may be kept in the heater overnight in case the sections are prepared the previous day to the machine start-up.

INSTRUCTIONS FOR USE OF THE EQUIPMENT

Once the MD STAINER and the computer are on, we must access the following icon to run the software which allows controlling the platform:

Note: *it is always recommended to turn on* ***the instrument first and the computer afterwards****.*

In the main screen, we have available all the functions to program a staining cycle that we describe in detail below following the daily work order:



1st PREPARE LABELS

In order to prepare the labels, click on **"Prepare Labels"** and select the option create. The fields available will be filled in, which display the information of each sample. Then, assign the techniques and click on *"Save"*. Once all the labels have been created for the necessary techniques, print them and place them in the corresponding preparations.

2nd PLACE THE PREPARATIONS IN THE EQUIPMENT

The preparations must be placed in the equipment as the incubation modules are arranged, that is, horizontally, with the label outwards the equipment, by pressing them slightly so that they remain stuck in the support points of each module.

3rd SCAN SLIDES

It allows the scanning of the preparations previously labelled and placed in the equipment.

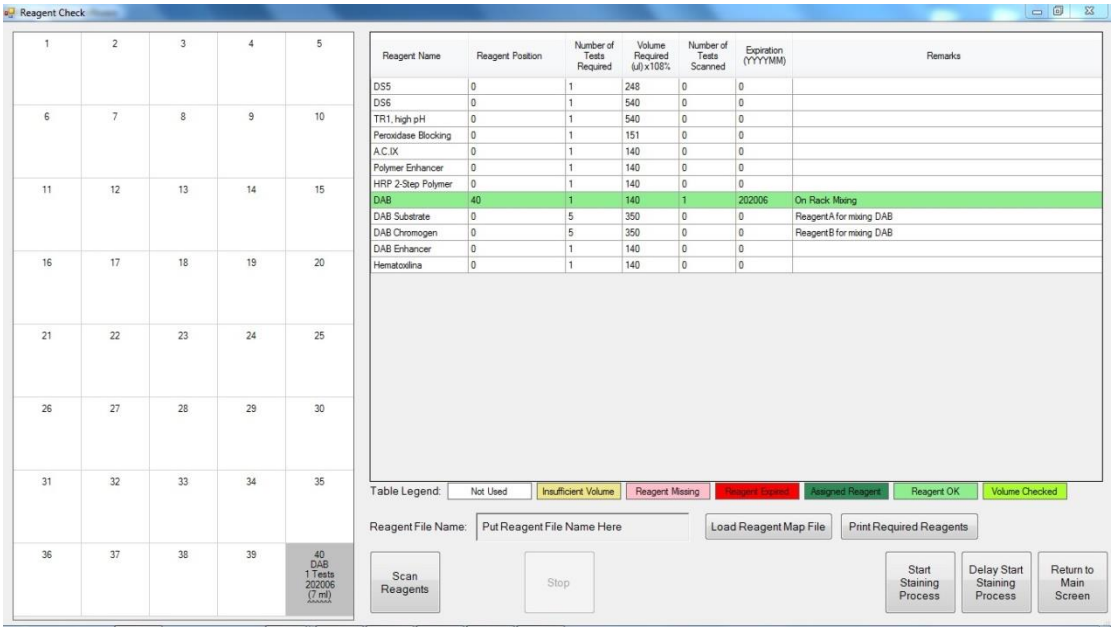
- If we choose this option without selecting any of the positions manually, the equipment will scan the 36 positions automatically.

- If the desired positions are previously selected with the left button of the mouse in the slide map (they can be clicked and dragged), the platform will scan the selected positions only.

  If you do not have any labels or the determinations to perform do not require them, the system allows the placing of the preparations and the assignment of the respective protocols manually by means of the option "***Assign Protocols".*** To do this, select one position of the corresponding slide with the left button of the mouse (or more than one) and click on the button "Assign protocols".

4th SCAN REAGENTS

Once the techniques to perform have been scanned and assigned manually, the user must Scan Reagents. When clicking on this option, a screen will appear, displaying all the necessary reagents to complete the process, as well as the volume and number of tests necessary for each one.



Once the reagents have been placed in the rack, click on ***"Scan Reagents"*** and the reading process will start automatically.

The reagents scanned will be shown in their corresponding position in the rack, in the reagents map on the left (positions 1-40) and it will display the following information:

- Reagent name.
- Current number of tests.
- Expiration date.
- RFID labels identification

During this process, the system can identify and inform about the following possible issues during programming by means of different colors:

White - Not used. There is no techniques that this reagent needs.
Yellow - Insufficient. Insufficient reagent volume.
Pink - Missing reagent. The system did not detect this reagent in the rack

Red – Expired. It expired.

In any case, the technician must **replace and/or place the expired, missing or insufficient-number-of-tests reagents** and rescan.

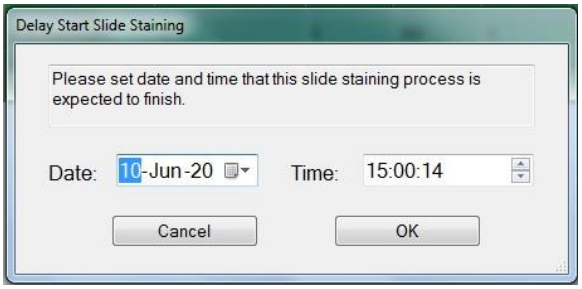
Important note: In position 40, an empty vial must always be placed so that the instrument can prepare the DAB. In case the stains require another type of chromogen, the system will indicate the position where the corresponding empty vial must be placed.
This bottle must not be reused, a new one must be placed every staining cycle.

Once all the scanned reagents are shown in green, everything is ready to start the staining process.

5th START STAINING PROCESS

There are two options to start the staining process:

1. ***Start staining process***. In this case, the staining will start immediately.
2. ***Delay Start***. It allows postponing the start. The user must specify in the system the date and time the process must end. (important to consider the values AM/PM).



Tip: *For overnight cycles, use the option Delay Start so that the tissues do not dry once the process is finished.*

When the process starts, the user must make sure that both the **washing buffer and the waste capacity are sufficient**. To do this, the system displays a screen with the necessary total volume for both and once checked, the user must press **OK** to start the process.

6th FINISH STAINING PROCESS

The system will mark in light green the slides that completed successfully the staining and will name them as "**Finished"**. After completing the last step of the process, the system unlocks the instrument's door and will generate a notification with alarm for the user: **"Process successfully completed"**.

Once the whole staining process has finished, the modules used will remain closed, keeping all the preparations hydrated. In order to take the preparations from the instrument, press "Ok" in the notification **"Process successfully completed"** and press ***Load / Unload Slides*** *to open the modules.*

It is recommended to take the preparations out of the instrument for a cuvette with washing buffer and keep in that buffer for 3 minutes to remove any precipitate that could have formed during the staining process.

OTHER OPTIONS AVAILABLE (Not necessary in the routine use of the equipment)

Load / Unload Slides

This function, which is used to open and close the incubation modules and thus allows the load or unload of slides at the beginning and end of the staining process. Nonetheless, once the system is started, all the modules remain open automatically so that the user can introduce the slides without selecting this option.

Automatic start

To start a preset program.

System

This section displays all the configuration options to control, check the system, create and modify reagents and protocols... For more detailed information about this section, please refer to the User manual.

Clean screen

It allows cleaning the screen once the process has finished or when the user assigns a protocol by mistake.

Exit

It allows exiting the software.

CLEANING AND PREVENTIVE MAINTENANCE

MANDATORY CLEANING RECOMMENDATIONS

It is necessary to follow the mandatory cleaning recommendations on a recommended timely basis to maintain the reliability, shelf life of the system and the staining quality.

1. CLEANING OF THE MODULES AND HEATERS (DAILY)

The modules must be cleaned every day to remove any reagent remains and avoid any accumulation in the instrument.

- Unload all the slides from the system.
- **It is recommended to use smooth, blotting paper** that cleans the excess that remains after the staining cycle both on the modules and on their surroundings.

2. CLEANING AND INSPECTION OF THE INCUBATION CHAMBERS (DAILY)

In order to avoid the accumulation of salts and reagent waste during the staining cycle, the chambers must be cleaned every day. During the cleaning, you must look for any cracks, leaks or degradation on the chamber surface.

To do this, after every staining cycle, clean the surface of the chambers with alcohol swabs. It is not necessary to remove the chambers from the instrument.

If, during the cleaning, you spot any anomalies on the chamber such as cracks and/or degradation when pressing the chamber, you must replace it with a new one.

3. THOROUGH CLEANING OF THE CHAMBERS (WEEKLY)

Besides this daily, weekly cleaning, or every 3-5 staining cycles, you must clean **the chambers thoroughly**. This cleaning is done as follows:

- Remove the chambers from the instrument.
- Introduce them in a 1/10 diluted bleach solution. Keep them there for 30 min.
- After the specified time, take the chambers from the bleach solution and rinse well with water, preferably distilled water.
- Dry them with blotting paper.

If, after the cleaning, any of the chambers is still dirty, you must replace it with a new one.

4. REPLACEMENT OF ALL THE INCUBATION CHAMBERS

The incubation chambers have an estimated shelf life of 40 cycles.

This number of cycles correspond approximately to two weeks of use for a client that performs two working daily cycles (Morning and Night). Therefore, all chambers must be replaced with new ones on a fortnightly basis in these cases.

For clients with a lower working volume, the replacement of all chambers must be calculated according to the shelf life time described above.

5. CLEANING OF THE UPPER SURFACE OF THE WASHING STATIONS AND THE PROBES Z1/Z2 (WEEKLY)

Salt and reagent remains can accumulate in the upper surface of the washing stations and the probes Z1/Z2 with the prolonged use of the instrument. Clean the waste with a cotton/towel moistened in alcohol.

6. VISUAL INSPECTION OF THE MODULES, ROBOT AND VERIFICATION OF THE CORRECT PERFORMANCE (WEEKLY)

Contact your MD-Stainer supplier for any anomalies detected or supply of spare parts and consumables.

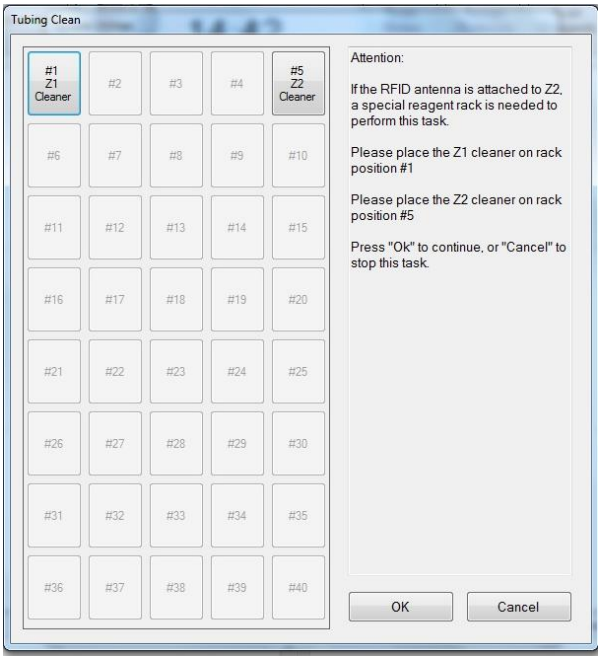
7. CLEANING OF SUCTION/DISPENSING TUBES (FORTNIGHTLY OR EVERY 400 DETERMINATIONS)

The tube corresponding to Z2 can accumulate DAB waste, Hematoxylin or other reagents with the prolonged use of the instrument. It is recommended to inspect regularly and visually both tubes Z1 and Z2 and perform the cleaning if necessary.
The system itself also tracks the number of determinations performed before the cleaning cycle. By default, 400 determinations are programed. Once this figure is reached, the system will automatically request the tube cleaning.

To do this:

- Load the reagent rack with the cleaning solutions for Z1 and Z2
- Place the rack in the system.
- Connect the fluid system (Washing buffer).
- Access to “System” in the main screen, and click on “Tube Cleaning”.
- Follow the instructions below:
    a) Place the cleaning solution Z1 in the # 1 position of the reagent rack as shown in the image.
    b) Place the cleaning solution Z2 in the # 5 position of the reagent rack as shown in the image.

c) Click on “OK” to start the tube cleaning.



Both the probes Z1 and Z2 aspirate 5 ml of cleaning solution for each one from the vials placed in their respective position of the reagent rack. Then, the system will show a 20-minute countdown.

After 20 minutes, the instrument will initialize the system automatically and will purge the waste of the cleaning solutions. Click on “Cancel” anytime to purge the tube waste immediately.

Preventive maintenance by the Technical Service

The maintenance will be carried out for the whole system by a trained technician. Contact your MD-Stainer supplier to program the maintenance.